Distribution and Modification of Sorption Sites in Amphiphilic Calixarene-Based Solid Lipid Nanoparticles from Hyperpolarized ^{129}Xe NMR Spectroscopy

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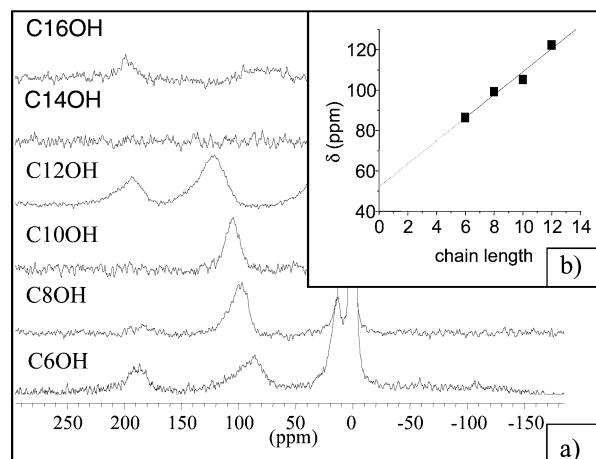
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Organic nanoparticles of intermediate size between molecules and microcrystals offer unique opportunities for a variety of applications, especially if they can be dispersed in water to give stable colloidal suspensions.[1,2] However, challenges exist both in developing procedures for obtaining reproducible products and for their characterization.
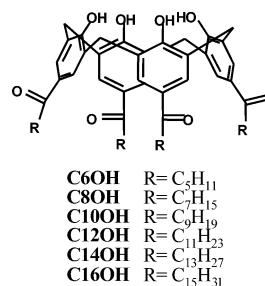
The calixarenes are a versatile class of macrocyclic compounds that have been studied extensively, both as host materials and as platforms for the synthesis of designed specific receptors.[5] Amphiphilic calixarenes obtained by Friedel−Crafts acylation of the parent calix[4]arene[6] represent a promising class of material for this purpose, since they retain their ability to complex small organic molecules, have specific interactions with ions at the air−water interface,[7] and self-organize as solid lipid nanoparticles (SLNs) in water.[8] The use of SLNs as a colloidal transport system is of great interest because of their high stability and high encapsulation loads due to their matrix structure.[9] However, since SLNs are noncrystalline, their detailed structure, site distribution, and mode of action remain largely unknown. Here, we use NMR spectroscopy with hyperpolarized xenon[10,11] to obtain such information on SLNs while revealing a simple technique for improving site accessibility and illustrating the utility of the HP Xe NMR approach[12] to small (∼milligram) samples of biomaterials.

Colloidal suspensions of amphiphilic calixarenes (Chart 1) were prepared as before[8a] to give SLNs, and these were freeze-dried to give a product of reasonably monodisperse particles of mean diameter 150 nm. The room-temperature spectra of amphiphilic calixarenes, with a chain length ranging from six to 16 carbons, obtained in situ in the NMR probe with HP Xe produced in a continuous flow system are shown in Figure 1a. The four observed resonances at 0, ∼20, ∼80−130, and ∼190 ppm can be assigned to free Xe, Xe in the interparticle space, Xe interacting with calixarene host cavities, and Xe solubilized in the hydrophobic chains. Compared to crystalline powders of the amphiphilic calixarenes, the spectral intensity is much greater for SLNs because of the higher surface area of this material (see Supporting Information). An increase in the shift of xenon included in the host cavity with the number of carbons of hydrophobic chains suggests reduced void space for xenon in the host cavity of calixarenes[13] as the chain length increases. The observed shift is proportional to the chain length of the material (Figure 1b) and on extrapolation to zero gives a chemical shift of 52 ppm, which is very close to the value previously obtained for the short-chain analogue, *p-tert*-butylcalix[4]arene (∼60 ppm).[14] It was known previously that for C6OH, C8OH, C10OH, and C12OH the chains could fold *over* the cavity,[7] thus affecting cavity access. From the Xe results, the

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Figure 1. (a) Static ^{129}Xe NMR spectra recorded under continuous flow of hyperpolarized xenon at an effective Xe pressure of 7 Torr and $T = 293$ K. (b) Plot of the chemical shift of Xe in the host cavity versus the chain length of the amphiphilic calixarenes
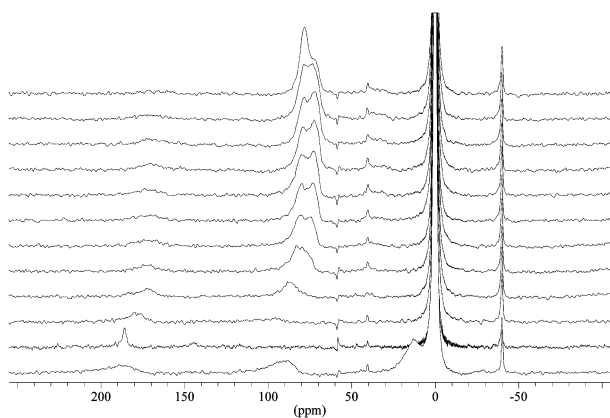
Chart 1. Chemical Structure of Amphiphilic Calixarenes

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C6OH	R= C_5H_{11}
C8OH	R= C_7H_{15}
C10OH	R= C_9H_{19}
C12OH	R= $C_{11}H_{23}$
C14OH	R= $C_{13}H_{27}$
C16OH	R= $C_{15}H_{31}$

dependence of accessible cavity space on chain length for C6OH−C12OH suggests that the hydrophobic chains do not just fold over, but also into the cavities, with perfect filling of the void space for C14OH and somewhat less efficient filling by the longer chain of C16OH. The amount of Xe associated with the lipid chains[10c] ($\delta \approx 190$ ppm) is rather variable, not unexpected for chains which likely exist in varying degrees of order for different chain lengths.

To test this material for sorbent properties and its interaction with small organic molecules, methylene chloride was added into the Xe/N$_2$/He flowing gas mixture from the polarizer. Figure 2 shows ^{129}Xe magic angle spinning (MAS) NMR spectra before and after a pulse of methylene chloride was applied to C6OH. When methylene chloride and xenon were adsorbed together (CH$_2$Cl$_2$−Xe on), only a small narrow signal at ∼190 ppm is observed, which can be assigned to xenon dissolved in a relatively mobile phase of lipid chains. Spectra recorded after the pulse of methylene chloride

Figure 2. In situ transformation of C6OH SLNs (bottom) after a pulse of methylene chloride (next to bottom) as monitored by continuous flow hyperpolarized ^{129}Xe MAS NMR. Consecutive spectra were recorded every 4.5 min at room temperature. Sharp signals at ± 40 ppm are spinning sidebands.
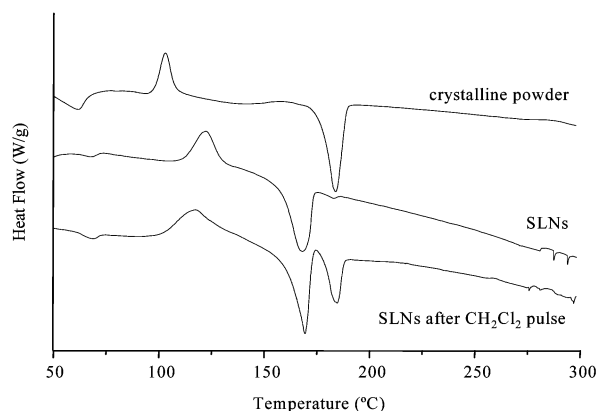
Figure 3. Differential scanning calorimetry traces for *p*-hexanoyl calix-[4]arene C6OH.

clearly indicate the return of xenon to the host cavity as methylene chloride is stripped out; however, the environment after CH$_2$Cl$_2$ adsorption has changed distinctly. Instead of a single asymmetric signal, we now see two closely spaced and more shielded lines ($\delta_{iso} = 78.6$ ppm and $\delta_{iso} = 73.6$ ppm), indicating that xenon is present in two slightly inequivalent sites.

The intensity increase with time can be associated quite naturally with the release of methylene chloride molecules. Increased deshielding of the signals suggests Xe samples a somewhat larger void volume. It is interesting that the total recovered Xe signal intensity after the CH$_2$Cl$_2$ pulse is considerably greater than the initial one, indicating improved accessibility of the host cavities. Splitting of the original signal into two peaks is similar to the case of *p-tert*-butylcalix[4]arene, where it was previously shown that two inequivalent xenon sites were defined by the statically disordered host *tert*-butyl groups.[13]

Figure 3 shows the DSC traces for the crystalline powder, SLNs, and SLNs after the CH$_2$Cl$_2$ pulse of C6OH. The thermogram of C6OH powder shows an exothermic change at 103 °C and an endothermic peak at 183 °C. The exothermic change corresponds to crystallization. The thermogram of C6OH-based SLNs shows the same transitions at 130 °C and 169 °C, respectively. In the case of SLNs after treatment with methylene chloride, a broad exothermic peak is observed at 117 °C as well as two endothermic peaks at 169 °C and 184 °C. This thermogram corresponds approximately to a superposition of those obtained for C6OH

powder and C6OH-based SLNs, suggesting the presence of two distinct phases.

In regard to these results, it is reasonable to suggest that treatment of SLNs with methylene chloride results in a phase transition between a state where hydrophobic chains are self-included into adjacent calixarenes and a state where the chains are excluded from the host cavity. Such a model is confirmed by the fact that similar changes are seen when other small molecules (DMSO, CHCl$_3$, o, *p*-xylene, CCl$_4$) are adsorbed, but not when larger molecules such as menthol are used. The modifying molecules must be able to occupy the cavity preferentially to displace the self-including hydrocarbon chains. The resulting material is a composite with a partially ordered shell that has a more accessible void space on top of the original amorphous disordered core.

In summary, this is the first ^{129}Xe NMR study of SLNs, illustrating three key features. (i) Host cavities present at the surface of the particles are still accessible to small atoms (Xe) and organic molecules (methylene chloride, etc). This is of considerable interest to understand the drug release mechanism(s) as molecules can be located in three areas: adsorbed on the surface, complexed with the host molecules, and entrapped in the SLN matrix, with each giving a different release process. (ii) The host cavities are loaded with the hydrophobic chains of adjacent calixarenes, thus hindering complexation of guest molecules. (iii) Although this may constitute the way that these molecules self-organize in water, it is possible to reorganize the surface of the material by flowing vapors of guest molecules that can displace the included chains from the host cavity, thus giving increased void space for sorption.

Supporting Information Available: Experimental details and ^{129}Xe NMR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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